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Detection of a well defined clonal lineage of *Pasteurella multocida* associated with fowl cholera by real-time PCR

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Fowl cholera is a disease of considerable economic importance to the poultry industry caused by *Pasteurella multocida*. The disease may manifest itself as peracute or acute systemic infections associated with high morbidity and mortality although chronic infections with mild symptoms and low mortality are reported. Outbreaks of fowl cholera also affect the wild fauna. A special lineage of P. multocida is associated with outbreaks around the Baltic Sea and has subsequently spread to commercial poultry (Christensen et al. 1998; Eigaard et al. 2006; Bisgaard et al. 2008a). Recently a definitive DNA sequence based typing system (MLST) has been developed for P. multocida and results generated with this method showed a specific MLST type to be associated with isolates of P. multocida from fowl cholera (Bisgaard et al. 2008b). However, the method is both time consuming and expensive and therefore the aim of this study was to develop a Real-Time (RT) PCR method for specific detection of the clonal lineage involved in fowl cholera. Part of the housekeeping gene pgi, glucose-6-phosphate isomerase, was amplified and sequenced. Single-nucleotide polymorphisms (SNP) were analysed using the software Minimum SNP. Primers were subsequently designed using software Primer3. RT-PCR was run on a MxPro3000 (Stratagene). We sequenced an internal fragment of the pgi gene in 250 isolates of P. multocida as part of testing a MLST scheme for P. multocida. One allele of the gene was only found among isolates belonging to the clonal lineage involved in fowl cholera. The software Minimum SNP detected two single-nucleotide polymorphisms in the gene where the fowl cholera associated allele demonstrated a T-A basepair, while all other alleles demonstrated C-G. Primers were designed to anneal at 3' over one the specific region. An intentional mismatch was incorporated to increase allele specificity. The primers were tested blind with 12 isolates where six belonged to the fowl cholera clonal lineage. The RT-PCR was found specific for isolates of P. multocida belonging to the type associated with fowl cholera in the area of the Baltic Sea. RT-PCR allows identification of the clonal lineage within three hours starting from colonies on the primary plates.